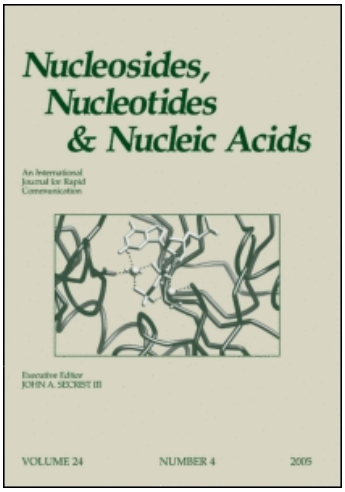

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The Role of Hydrophobic Interactions in Catalysis of RNA Cleavage by 1,4-Diazabicyclo[2.2.2]-Octane Based Artificial Ribonucleases

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ABSTRACT

Molecular interactions of RNA cleaving compounds–conjugates of 1,4-diazabicyclo-[2.2.2.]-octane substituted at the bridge position with tetradecamethylene fragment and imidazole were investigated using light scattering and small angle x-ray scattering methods. The compounds are known to efficiently cleave RNA and one source of the activity could result from micellar catalysis. It was found that the compounds indeed are capable of forming complex aggregates in solution. However, maximal efficacy of RNA cleavage by the conjugates is observed at concentrations well below the concentration required for micelle formation.

Key Words: RNA cleavage; Light scattering; Small angle x-ray scattering; Artificial ribonuclease; Micelle formation.

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INTRODUCTION

Recently synthetic ribonuclease mimics–conjugates of 1,4-diazabicyclo[2.2.2]octane (DABCO) substituted with a tetradecamethylene fragment at the bridge position and imidazole have been designed.[1,2] The compounds of general formula ABLkCm were built of four main domains: tetradecamethylene aliphatic group (A), RNA binding domain B (DABCO residue), flexible linker (Lk) and RNA-cleaving domain Cm, containing an imidazole residue. The compounds ABLkC3 (Fig. 1) displayed the highest ribonuclease activity. It was found that the aliphatic fragment contained in the structure of the synthetic ribonucleases is important for activity of the compounds.[2] This unexpected effect might be attributed to micellar catalysis: one could expect that micelle formation could provide a cationic surface with varying density of catalytic groups capable to bind and cleave RNA. Therefore, we investigated micelle formation in ABL3C3 solutions using small angle X-ray scattering and light scattering techniques.[3] It was found that indeed, the compounds can form micelle under experimental conditions, however the micelle formation is not the source of the ribonuclease activity of the catalyst.

EXPERIMENTAL

The compound ABL4C3 was synthesized by Dr. D. Konevetz (this institute), $tRNA^{Phe}$ was a generous gift of Prof. G. Keith (Strasbourg, France). Total tRNA from *E. coli* was purchased from ''Vector,'' Russia. Small-angle X-ray scattering (SAXS) roentgenograms were registered on diffractometer with Kamera Kratky (Siemens, Germany) by the method of step-by-step scanning using a goniometer and X-ray scintillation detector.[3] Small-angle roentgenograms were measured in the angular range: $h = 0.013 \div 0.35$ $Å^{-1}$, where $h = 4\pi \sin(\theta)/\lambda$; 2θ is scattering angle. A special thermostated (22 and 37°C) quartz capillary cuvette (1.0 mm in diameter) with a wall thickness of 0.01 mm was used. The radiation wavelength (λ) was 1.54 Å. To smooth X-ray data the small-angle X-ray roentgenograms were corrected taking into account background scattering, adsorption, and collimation. SAXS data were mathematically processed by executing special computer programs and algorithms described earlier,[4] and optimization programs.[5] Light scattering intensity was measured using Shimadzu RF-6000 spectrophotometer (Japan) with 1×1 cm quartz cell thermostated at 22°C. Slits were 6 nm for excitation and 14 nm for emission. Excitation and emission intensity were measured at $\lambda = 430$ nm (at $h = 0.02066$ nm^{-1}). Corrections were made with regard to the inner filter effect, compound dilution, and Raman scattering intensity.[6] The results were plotted as mean ± standard deviation of at least 3 different

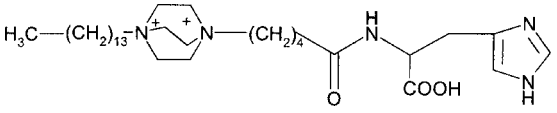

Figure 1. Artificial ribonuclease ABL4C3. (From Ref. [1].)

experiments for each analyzed sample. The differences between samples were analyzed by Student's t-test, $P \leq 0.05$ was considered statistically significant.

$tRNA^{Phe}$ was 5′-end labeled with [P^{32}]-ATP by standard procedure.[7] RNA cleavage was performed in 50 mM Tris-HCl buffer, pH 7.0, containing 0.2 M NaCl, and 0.1 mM EDTA. The cleavage products were analyzed by 12% PAAG/8M urea gel electrophoresis using TBEx1 (100 mM Tris-borate, pH 8.3, 0.9 mM EDTA) as running buffer.

RESULTS AND DISCUSSION

Recently we investigated cleavage of RNA with artificial ribonuclease ABLkC3 (Fig. 1). The concentration dependence of $tRNA^{Phe}$ cleavage was bell-shaped with maximum at 0.5 mM,[2] which is not typical for others DABCO and imidazole containing artificial ribonucleases lacking hydrophobic groups.[8,9] One explanation of the bell-shaped concentration dependence displayed by the compounds ABLkC3 could be micelle formation. Therefore, we investigated formation of micelles in solutions of ABL4C3 and assayed ribonuclease activity in these solutions.

Light Scattering (LS) was used to follow micelle formation in the solutions of ABL4C3 and to measure average size of particles (parameter I_{90}).[4] The dependence of average particle size (I_{90}) on concentration of ABL4C3 is bell-shaped with maximum at

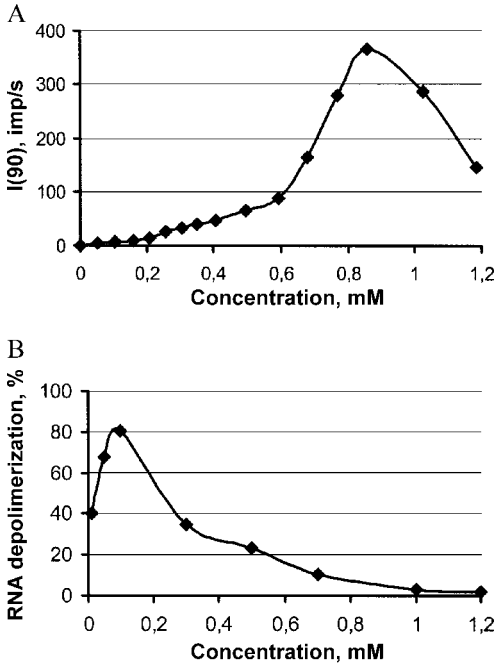

Figure 2. Dependence of particle size (I_{90}) on concentration of ABL4C3 in solution (LS roentgenogram) (A) and dependence of tRNA depolymerization reaction on concentration of ABL4C3 (B).

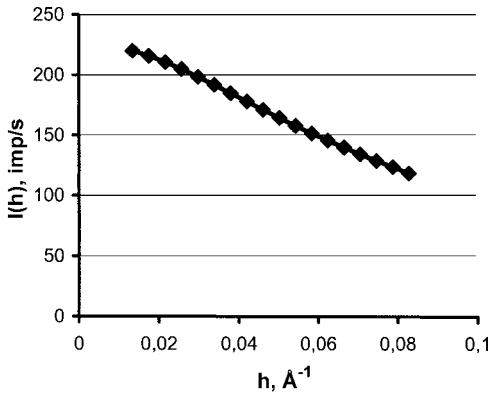

Figure 3. SAXS roentgenogram of total *E. coli* tRNA dissolved at concentration 5 mg/ml in 50 mM Tris-HCl buffer, pH 7.0, containing 0.2 M NaCl, 0.1 mM EDTA.

0.9 mM concentration of the compound. This type of curves significantly differs from the S-shaped curve typical for concentration dependencies of micelle formation. Investigation of RNA cleavage in the ABL4C3 solutions (Fig. 2B) and micelle formation as functions of the compound concentration clearly demonstrated that the catalysis is not related to the micelle formation. The RNA cleavage rate was maximal at concentration of the catalysts well below the critical concentration required for micelle formation.

We used small angle x-ray scattering (SAXS) as a direct method to follow RNA depolymerization in the presence of ABL4C3. Figure 3 shows an experimental SAXS

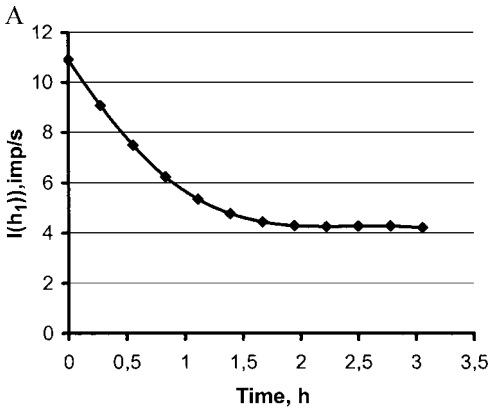

Figure 4. Time dependence of RNA-cleavage by compound ABL4C3 monitored by intensity of X-ray dispersion from particles in the initial point of SAXS roentgenogram (A) and 15% denaturating PAAG (B). Incubation time is indicated at the top of radioaftograph. Assay conditions: the samples were prepared in 50 mM Tris-HCl buffer, pH 7.0, containing 0.2 M NaCl and 0.1 mM EDTA. The concentration of ABL4C3 and total *E. coli* tRNA was 0.5 mM and 0.05 mM, respectively. Depolymerisation of tRNA in denaturating PAAG was visualized using 5′-[^{32}P]-tRNA^{Phe}.

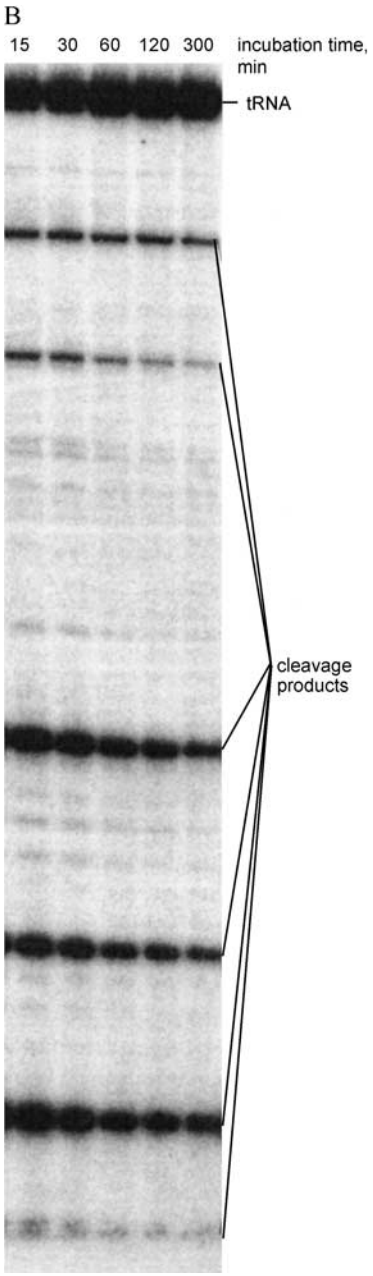

Figure 4. Continued.

roentgenogram of total *E. coli* tRNA samples at concentration 5 mg/ml without the cleaving compound. The average value of inertia radius (R_g) of the RNA calculated from the data presented in Fig. 3 is: $R_g = 21.3 \pm 0.6$ Å. The dispersion intensity at zero angle ($h = 0$) is $I(0) \sim n \cdot M^2$, where n and M are the number and the average

molecular weight of molecules involved in dispersion, respectively.[3] Small-angle X-ray scattering measures the average size of biopolymers in the sample [parameter $I(h_1)$]. In Fig. 3 it is seen, that the initial part of SAXS roentgenogram has negligible angle of inclination in coordinates I(h), h. This allowed us to estimate the intensity of X-ray dispersion of particles at the initial point as $I(h_1) \approx I(0)$.[10] The dependence of $I(h_1)$ on the reaction time estimated by this approach is shown in Fig. 4A. Thus, we observed kinetics of decreasing of RNA size due to cleavage by the compound ABL4C3.

In parallel, RNA cleavage was assayed electrophoretically in the experiments with solutions containing 5′-[^{32}P]-tRNA^{Asp} and cold total RNA preparations (Fig. 4B). It is seen that decreasing of the particles size [$I(h_1)$] of total *E. coli* tRNA correlates well with time course of cleavage monitored by electrophoresis.

Results of the present study evidence that cleavage of RNA by synthetic ribonuclease ABL4C3 occurs with maximal intensity at concentrations of ABL4C3 well below the concentration needed for micelle formation. Therefore, the role of the hydrophobic structure in the catalysis of RNA cleavage by ABL4C3 remains to be elucidated.

ACKNOWLEDGMENTS

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